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Synthesis and biological evaluation against *Leishmania amazonensis* of a series of alkyl-substituted benzophenones

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ABSTRACT

Nine *O*-alkyl and *O*-prenyl derivatives were synthesized from commercial 2,4-dihydroxybenzophenone, 4,e4,4′-dihydroxybenzophenone and were evaluated for their leishmanicidal activity against promastigote forms of *Leishmania amazonensis*, as well their toxicity in murine macrophages. All derivatives exhibited better biological activity than their hydroxylated benzophenones precursors, and new compound **LFQM-123** (**3c**) was 250-fold more active than its precursor 4,4′-dihydroxybenzophenone (**3**). Moreover, some of the results were comparable to the standard drug Amphotericin B, suggesting that the increase in lipophilicity could facilitate protozoa membrane permeation. In this study we confirmed that benzophenone derivatives exhibit leishmanicidal properties, with relatively low toxicity, and thus could be exploited as promise prototypes for the design and development of new drug for the treatment of leishmaniasis.

1. Introduction

Leishmaniasis is a parasitosis transmitted by several species of the protozoan genus *Leishmania* and is endemic in 88 tropical, subtropical countries around the world. Recent rough estimates point for a global prevalence of 12 million infected people and other 350 million at risk. This infectious disease is characterized by both diversity and complexity, due to different clinical syndromes that could be developed for more than 20 leishmanial species, transmitted to humans by more than 30 different species of phlebotomine sandflies.[1] An important point to be considered is that in most of cases, infected people live in countries with sanitary and fragile governmental health systems, which are close related to the high taxes of morbidity and mortality of the disease.

After transmission from the insect, all species of *Leishmania* change from the flagellar promastigote form to the amastigote form and are internalized mainly in the macrophages. Then, they can multiply and survive in the secondary phagolysosomes and infiltrate to infect other surrounding macrophages.[2] The disease occurs in four main clinical forms: cutaneous leishmaniasis, muco-cutaneous leishmaniasis, visceral leishmaniasis (VL) and diffuse cutaneous leishmaniasis. Visceral leishmaniasis, also known as kala-azar, is caused *Leishmania donovani* and *L. infantum* and is the most serious infection form of the disease. In VL, the patient generally presents symptoms of persistent infections, including fever, appetite and weight loss, hepatomegaly, hypergamaglobulinemy, weakness, fatigue, and death if untreated[2,3] *Leishmania brasiliensis* is responsible for the most cases of muco-cutaneous leishmaniasis, one of the most common clinical manifestation in Brazil and other tropical countries. *Leishmania amazonensis* is a member of the *Leishmania Mexicana* complex and the etiological agent for a broad-spectrum of leishmaniasis in South American countries.[4] In Brazil, *L. amazonensis* infection can cause two distinct clinical forms of cutaneous leishmaniasis: localized cutaneous leishmaniasis and diffuse cutaneous leishmaniasis.[5]

Chemotherapy is the current basis for the treatment of leishmaniasis, and effective anti-leishmanial vaccines are still under study and development.[6] Pentavalent antimonial drugs are the first choice treatment for leishmaniasis,[2] and resistance arise is frequently a problem in endemic areas. Amphotericin B and pentamidine are the second choice drugs, and have their therapeutic

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effectiveness limited by high toxicity and difficulties in administration.[2] In this context, and due to the restricted therapeutic alternatives and high toxicity of the current medicines, the search in biodiversity for new active compounds and extracts against *Leishmania* is a current field in natural products and medicinal chemistry.[6,7] In a recent communication, our group showed that natural polyprenylated benzophenones like guttiferone-A, 7-*epi*-clusianone and garciniaphenone isolated from the fruits of *Garcinia brasiliensis*, exhibited leishmanial activity against amastigote and promastigote forms of *L. amazonensis*, and 7-*epi*-clusianone was the most active compound.[8] In another work, Luque-Ortega et al.,[9] synthesized a series of benzophenone salts that revealed that the presence of more lipophilic substituents clearly contribute for the increase in leishmanicidal activity. In this work, we report the synthesis of a series of methyl, butyl and prenyl-derivatives of 2,4-dihydroxybenzophenone (1), 4-hydroxybenzophenone (2) and 4,4′-dihydroxybenzophenone (3) and their biological evaluation against Leishmania and cytotoxicity.

2. Materials and methods

2.1. Experimental

The reactions were monitored by thin-layer chromatography (TLC) performed on silica gel 60 F254 (Merck®). Melting points (in °C) were determined using a Mettler melting point apparatus (Mettler-Toledo, Leicester, UK) and are uncorrected. Chromatography was performed on silica gel 60, 0.063–0.200 mm, (ACROS®). Elemental analysis was performed using a TruSPEC(CHN-S) Analyzer by Leco Instruments. NMR spectrum was recorded on a BRUKER Avance DPX 200 or BRUKER Avance DRX 400 spectrometers. Chemical shifts are reported in parts per million (ppm) with reference to tetramethylsilane (TMS). The coupling constants are reported in hertz (Hz) and signal multiplicities are reported as singlet (s), doublet (d), doublet of doublet (dd), mulitplet (m).

2.1.1. General procedure for conduction of O-alkylation reactions

To a stirred solution of hydroxylated benzophenones (1.0 mmol) in dimethylformamide/acetone (1:2 v/v) was added potassium carbonate (2.0 equiv) and alkyl chloride or bromide (1.5–3.0 equiv) at room temperature. The reaction mixture was kept at 60 °C monitored by TLC up to total consumption of starting benzophenone. Then, reaction mixture was partitioned between ethyl acetate and H_2O, and the organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude residue was purified by silica gel column chromatography using hexane and ethyl acetate (8.5:1.5) as eluent to give the alkylated benzophenone derivative (Table 1).

2.1.2. 2-Hydroxy-4-methoxybenzophenone[10]

Yellow crystalline solid. Mp 58 °C; IR (KBr) υ_{max} (cm^{-1}) 1256 (υ C–O–C); 1620 (υ C=O); 2864 (υ CH_3). CG–MS *m*/*z* (%) 105(5); 77(14); 228(25); 31(100). ^{1}H NMR ($CDCl_3$; 200 MHz) δ 3.8(s, 1H, H-8); 6.3(d, 1H, H-3, *J* = 2.4 Hz); 6.4 (d, 1H, H-5, *J* = 2.4 Hz); 6.5 (t, 1H, H-6, *J* = 2.4 Hz); 7.5(t, 2H, H-3′ and H-5′ , *J* = 0.8 Hz); 7.6 (d, 2H, H-2′ and H-6′, *J* = 2.0 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 56.9 (C-3); 108.6 (C-5); 114.4 (C-1); 129.5 (C-2′ and C-6′); 130.1 (C-3′ and C-5′); 132.7 (C-4′); 136.5 (C-1′); 139.5 (C-6′); 167.5 (C-2); 167.6 (C-4); 201.3 (C-7).

2.1.3. 2-Hydroxy-4-*O*-butylbenzophenone[11] (LFQM-116, 1b)

Pale yellow crystalline solid. Mp 71 °C; UV (EtOH; 0.1 %) λ_{max} (log ε) 324 (0.24); 289 (0.15) and 243 (0.21) nm. IR (KBr) υ_{max} (cm^{-1}) 2800 (υ C–H); 1624 (υ C=O); 1266 (υ C–O–C). CG–MS *m*/*z* (%): 105 (5); 77 (14); 228 (25); 31(100). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.98 (t; 1H, H-11, *J* = 7.3 Hz); 1.4 (sex., 1H, H-10, *J* = 7.2 Hz); 1.7 (q, 1H, H-9, *J* = 7.5 Hz); 4.0 (t, 1H, H-8, *J* = 6.5 Hz); 6.4 (d, 2H, H-3 and H-5, *J* = 2.2 Hz); 7.7 (d, 1H, H-6, *J* = 2.4 Hz); 7.5 (d, 2H, H-2′ and H-6′, *J* = 6.2 Hz); 7.7 (t, 1H, H-4, *J* = 4.0 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 16 (C-11); 17.4 (C-10); 21.8 (C-9); 70.7 (C-8); 96.8 (C-3); 102.7 (C-5); 113.9 (C-1); 130.1 (C-3′; C-5′); 129.7 (C-2′; C-6′); 129.5 (C-6); 135.3 (C-1′); 151.2 (C-2); 167.6 (C-4); 202.3 (C-7).

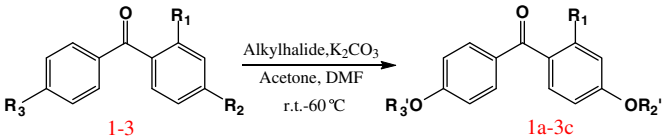
2.1.4. 2-Hydroxy-4-*O*-(3,3-dimethyl)-allylbenzophenone[12] (LFQM-117, 1c)

White crystalline solid. Mp 88 °C; UV (EtOH; 0.1 %) λ_{max} (log ε) 322 (0.25); 288 (1.70) and 243 (0.22) nm. IR (KBr) υ_{max} (cm^{-1}) 2914 (υ C–H); 1600 (υ C=O); 1271 (υ C–O–C). CG–MS *m*/*z* (%) 282 (1); 77 (5); 105 (5); 137 (6); 69 (50); 213 (60); 41 (100). ^{1}H NMR ($CDCl_3$; 200 MHz) δ 1.8 (s, 1H, H-11); 1.8 (s, 1H, H-12); 4.5 (d, 1H, H-8, *J* = 6.8 Hz); 5.4 (m, 1H, H-9); 6.4 (d, 1H, H-3, *J* = 2.0 Hz); 6.5 (d, 1H, H-5, *J* = 2.4 Hz); 6.6 (d, 1H, H-6, *J* = 2,4 Hz); 7.5 (m, 1H, H-4′); 7.6 (t, 2H, H-3′ and H-5′, *J* = 2.9 Hz); 7.7 (d, 2H, H-2′ and H-6′, *J* = 1.6 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 18.9 (C-11); 23.0 (C-12); 66.1 (C-8); 120.1 (C-9); 141.9 (C-10); 104.3 (C-3); 105.7 (C-5); 110.7 (C-1); 129.1 (C-3′ and C-5′); 129.7 (C-6); 163.8 (C-4); 130.1 (C-2′ and C-6′); 133.4 (C-4′); 137.4 (C-7).

2.1.5. 4-Methoxybenzophenone[10] (LFQM-118, 2a)

Yellow crystalline solid. Mp 183 °C; UV (EtOH; 0.1 %) λ_{max} (log ε) 416 (25); 288 (0.13), 252 (0.24); 224 (0.20); 204 (0.09) nm. IR (KBr) υ_{max} (cm^{-1}) 2968; 2935; 2845 (υ C–H);1649 (υ C=O); 1259

Table 1
Chemical structures for benzophenone derivatives **1a–3c**



Compound	R_1	R_2	R_3	R'_2	R'_3	Yield (%)
LFQM-115 (**1a**)	OH	OH	H	OCH_3	H	51
LFQM-116 (**1b**)	OH	OH	H	$O(CH_2)_3CH_3$	H	83
LFQM-117 (**1c**)	OH	OH	H	$OCH_2CHC(CH_3)_2$	H	46
LFQM-118 (**2a**)	H	OH	H	OCH_3	H	48
LFQM-119 (**2b**)	H	OH	H	$O(CH_2)_3CH_3$	H	63
LFQM-120 (**2c**)	H	OH	H	$OCH_2CHC(CH_3)_2$	H	51
LFQM-121 (**3a**)	H	OH	OH	OCH_3	OCH_3	91
LFQM-122 (**3b**)	H	OH	OH	$O(CH_2)_3CH_3$	$O(CH_2)_3CH_3$	73
LFQM-123 (**3c**)	H	OH	OH	$OCH_2CHC(CH_3)_2$	$OCH_2CHC(CH_3)_2$	76

(υ C–O–C). CG–MS *m*/*z* (%) 69 (50); 31 (90); 41 (100); 212 (100). ^{1}H NMR ($CDCl_3$; 200 MHz) δ 3.8 (s, 1H, H-8); 6.9 (d, 2H, H-3 and H-5, *J* = 8.6 Hz); 7.4 (d, 2H, H-2 and H-6, *J* = 6.8 Hz); 7.5 (t, 2H, H-3′ and H-5′, *J* = 6.5 Hz); 7.8 (t, 1H, H-4′, *J* = 8.8 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 56.9 (C-8); 117.7 (C-3; C-5); 129.2 (C-2; C-6); 131.4 (C-2; C-6); 129.8 (C-2′; C-6′) 138.7 (C-4′); 143.5 (C-1′); 169.4 (C-4); 201.5 (C-7).

2.1.6. 4-Butoxybenzophenone[11] (LFQM-119, 2b)

Pale yellow crystalline solid. Mp 65 °C; UV (EtOH; 0.1 %) λ_{max} (log ε) 416 (−0.0005); 290 (0.09); 226 (0.09); 204 (0.2) nm. IR (KBr) υ_{max} (cm^{-1}) 2962; 2926 (υ C–H); 1635 (υ C=O); 1247 (υ C–O–C). CG–MS *m*/*z* (%): 77(30), 105(25), 121(100), 254(1). ^{1}H NMR ($CDCl_3$; 200 MHz) δ 1.0 (t, 1H, H-11, *J* = 7.3 Hz); 1.5 (sex, 1H, H-10, *J* = 7.2 Hz); 1.7 (q, 1H, H-9, *J* = 8 Hz); 4.0 (t, 1H, H-8, *J* = 6.5 Hz); 6.9 (d, 2H, H-3 and H-5, *J* = 8.8 Hz); 7.7 (d, 2H, H-2 and H-6, *J* = 2.4 Hz); 7.5 (d, 2H, H-2′ and H-6′, *J* = 7.4 Hz); 7.4 (t, 2H, H-3′ and H-5′, *J* = 7.1 Hz); 7.5 (t, 1H, H-4′, *J* = 6.5 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 14.3 (C-11); 20.4 (C-10); 32.4 (C-9); 66.9 (C-8); 115.2 (C-3; C-5); 129.4 (C-2; C-6); 130.9 (C-3′; C-5′); 132.8 (C-4′); 133.6 (C-1′); 166.4 (C-4); 195.5(C-7).

2.1.7. 4-*O*-(3,3-Dimethyl)-allylbenzophenone (LFQM-120, 2c)

White crystalline solid. Mp 179 °C; UV (EtOH; 0.1 %) λ_{max} (log ε) 416 (−0.0005); 290 (0.02); 250 (0.01) nm. IR (KBr) υ_{max} (cm^{-1}) 2976; 2943(υ C–H); 1598 (υ C=O); 1255 (υ C–O–C). CG–MS *m*/*z* (%) 266 (1); 41(15); 105(20); 198(55); 121(65); 69(73). ^{1}H NMR ($CDCl_3$; 50 MHz) δ 1.7 (s, 2H, H-11 and H-12); 4.5 (d, 1H, H-8, *J* = 6.8 Hz); 5.5 (t, 1H, H-9, *J* = 6.8 Hz); 6.9 (d, 2H, H-3 and H-5, *J* = 8.8 Hz); 7.4 (d, 2H, H-2 and H-6, *J* = 7.2 Hz); 7.5 (d, 2H, H-2′ and H-6′, *J* = 7.2 Hz); 7.5 (d, 2H, H-3 and H-5, *J* = 7.2 Hz); 7.7 (t, 1H, H-4′, *J* = 3.3 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 19.5 (C-11); 27.1 (C-12); 66.3 (C-8); 120.2 (C-9); 139.1 (C-10); 115.5 (C-3; C-5); 129.4 (C-2; C-6); 133.0 (C-1); 131.2 (C-2′; C-6); 133.1 (C-4′); 163.8 (C-4); 195.9 (C-7).

2.1.8. 4,4′-Di-methoxybenzophenone[10] (LFQM-121, 3a)

Yellow crystalline solid. Mp 68 °C; IR (KBr) υ_{max} (cm^{-1}) 2924 (υ C–H); 1637 (υ C=O); 1253 (υ C–O–C). CG–MS *m*/*z* (%): 242 (1); 77 (10); 135 (25); 31 (100). ^{1}H NMR ($CDCl_3$; 200 MHz) δ 3.8 (s, 2H, H-8 and H-8′); 6.9 (d, 4H, H-3 and H-5, H-3′ and H-5′ *J* = 8.8 Hz); 7.8 (d, 4H, H-2 and H-6, H-2′ and H-6′, *J* = 8.8 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 56.7 (C-11); 114.4 (C-3; C-5;C-3′; C-5′); 131.7 (C-2; C-6; C-2′; C-6′); 133.4 (C-4; C-4′); 195.7 (C-7).

2.1.9. 4,4′-Di-butoxybenzophenone[11] (LFQM-122, 3b)

Pale yellow crystalline solid. Mp 151 °C; IR (KBr) υ_{max} (cm^{-1}) 2933 (υ C–H); 1637 (υ C=O); 1249 (υ C–O–C). ^{1}H NMR ($CDCl_3$; 200 MHz) δ 0.9 (t, 2H, H-1 and H-11′, *J* = 7.3 Hz); 1.4 (sex, 2H, H-10 and H-10′, *J* =7.3 Hz); 1.8 (q, 2H, H-9 and H-9′, *J* = 7.2 Hz); 4.0 (t, 2H, H-8 and H-8′, *J* = 6.4 Hz); 6.9 (d, 4H, H-3 and H-5, H-3′ and H-5′, *J* = 8.8 Hz); 7.0 (d, 4H, H-2 and H-6, H-2′ and H-6′, *J* = 8.8 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 14.6 (C-11 e C-11′); 19.9 (C-10 e C-10′); 31.4 (C-9 e C-9′); 68.7 (C-8 e C-8′); 114.6 (C-3; C-5; C-3′; C-5′); 131.3 (C-2; C-6; C-2′ and C-6′); 132.9 (C-1 e C-1′); 163.2 (C-4 and C-4′); 195.2 (C-7).

2.1.10. 4,4′-Di-*O*-(3,3-dimethyl)-allylbenzophenone[12] (LFQM-123, 3c)

White crystalline solid. Mp 99 °C. IR (KBr) υ_{max} (cm^{-1}) 2933 (υ C–H); 1637 (υ C=O); 1249 (υ C–O–C). Anal. C 78.91%, H 7.46%, calcd for $C_{23}H_{26}O_3$, C 78.83%, H 7.48%. ^{1}H NMR ($CDCl_3$; 200 MHz) δ 1.7 (s, 4H, H-11 and H-11′, H-12 and H-12′); 4.5 (d, 2H, H-8 and H-8′, *J* = 6.8 Hz); 5.5 (t, 2H, H-9 and H-9′, *J* = 6.5 Hz); 6.9 (d, 4H, H-3 and H-5, H-3′ and H-5′, *J* = 8.8 Hz); 7.7 (d, 4H, H-2 and H-2′, H-6 and H-6′, *J* = 8.8 Hz). ^{13}C NMR ($CDCl_3$; 50 MHz) δ 18.8 (C-11; C-11′); 26.4 (C-12; C-12′); 65.5 (C-8; C-8′); 119.6 (C-9; C-9′); 139.3 (C-10; C-10′); 114.6 (C-3; C-5 and C-3′; C-5′); 131.2 (C-2; C-6; C-2′ and C-6′); 132.7 (C-1; C-1′); 162.7 (C-4; C-4′); 195.0 (C-7). The confirmation of assignments was made by 2D-NMR experiments at 400 MHz (Supplementary data). This compound is first time described in the literature.

2.2. Biological evaluation

2.2.1. Culture of Leishmania and in vitro assay

Promastigote forms of *L. amazonensis* (MHOM/BR/71973/M2269) were grown on a 24-wells plate in Schneider's Drosophila medium (Sigma, USA) supplemented with 10.0% (v/v) heat-inactivated fetal bovine serum and 1.0% penicillin (10,000 UI/mL)/streptomycin (10.0 mg/mL) (Sigma, USA). Cells were harvested in the log phase, re-suspended in fresh medium, counted in Neubauer's chamber and adjusted to a concentration of 1×10^6 cells/mL. Compounds **1**–**3** and **1a**–**3c** were added to promastigote cultures, at 1×10^6 cells/mL, solubilized in dimethylsulfoxide (DMSO) (the concentration used was 0.6%, v/v in all wells) and incubated at 25 °C. After 72 h of incubation, the surviving parasites were counted in a Neubauer's chamber and compared with controls and DMSO in a concentration of 0.6% v/v, for the determination of 50.0% inhibitory growth concentration (IC_{50}). All tests were performed in triplicate and Amphotericin B (Eurofarma) was used as the reference drug.

2.2.2. Evaluation of cytotoxicity

For the cytotoxicity assay a suspension of 8×10^5 cells/mL of murine peritoneal macrophages, in RPMI 1640 medium, supplemented with 10.0% heat-inactivated fetal bovine serum and 1.0% penicillin (10,000 UI/mL)/streptomycin (10 mg/mL) were added to each well in 24-well plates, on the glass slides of 13 mm. The plates were incubated in a 5.0% CO_2 air mixture at 37 °C to adhesion of the cells. After 24 h, the non-adherent cells were removed by washing with the RPMI 1640 medium. Thus, several concentrations of compounds **1**–**3** and **1a**–**3c** (in the range of 0.05–160.0 μg/mL) were added to the wells containing the cells. All target compounds were solubilized in DMSO at a final concentration of 0.6% v/v and the plates were incubated for more 72 h. Then, the non-adherent cells were removed by washing with the RPMI 1640 medium and 50.0 μL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well at concentration of 5.0 mg/mL, followed by incubation for more 4 h, as described by Mossman.[14] After this, the medium was retired and 1 mL of DMSO was added to each well and it was homogenized for 15 min. Next, the absorbance of each individual well, minus the control value, was calculated in according to the next formula at 570 nm.

$$\%\text{inhibition} = \left(\frac{OD_{control} - OD_{drugs}}{OD_{control} \times 100}\right)$$

Each experiment was performed in triplicate, and the percentage of viable cells was calculated in relation to control cultures in the medium with just DMSO at the concentration of 0.6% v/v.

2.3. Statistical analysis

The leishmanicidal activities of compounds were expressed as the concentration that inhibits the growth of 50.0% of protozoan form. Statistical analysis was performed using nonlinear regression to obtain the values of IC_{50} and CC_{50} (cytotoxic concentration for 50.0% of macrophages), followed by variance analyses and Tukey's test. Differences were significant when the *p* value was lower than 0.05.

2.3.1. Evaluation of lipophilicity by Log*P* (oct/water)

Lipophilicity values were estimated through theoretical determination of Log*P* (oct/wat) by using the XLOGP3 program.[13] The additive model implemented in XLOGP3 uses a total of 87 atom/group types and two correction factors as descriptors. It is calibrated on a training set of 8199 organic compounds with reliable log*P* data through a multivariate linear regression analysis. Calculated lipophilicity expressed by Log*P* (oct/wat) of compounds **1**–**3** and **1a**–**3c** are showed on Table 2.

3. Results and discussion

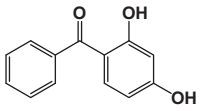
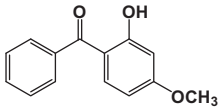
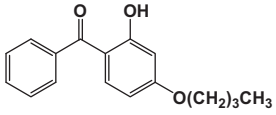
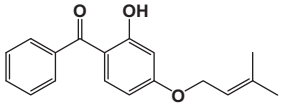
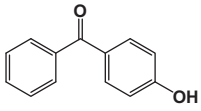
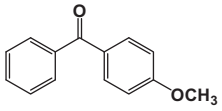
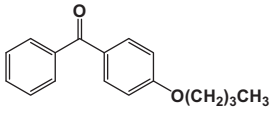
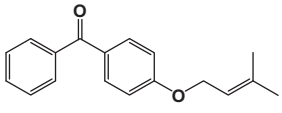
Considering that there is no vaccine available to prevent leishmaniasis, discovery of new effective and low toxic drugs for the treatment this neglected disease is an urgent need.[14] Despite natural and synthetic benzophenones are not commonly reported for their leishmanicidal properties, in the last years some works from literature have showed that many benzophenone-derivatives could be interesting for the development of new anti-protozoan agents.[15] In our work, we could confirm that benzophenone derivatives **1a**–**3c** with one or two hydroxyl groups in positions C-4 and/or C-4′ on the benzophenone skeleton exhibit a marked inhibitory activity against *Leishmania*, being more active than the starting hydroxylated benzophenones **1**–**3**, and with low toxicity.

Antiprotozoal activity of compounds **1**–**3** and **1a**–**3c** were evaluated in vitro against promastigote forms, and also for cytotoxicity in macrophages. Biological data evidenced that methylation, butylation and prenylation on the hydroxyl groups in C-4 and/or C-4′ position in the original hydroxybenzophenones **1**–**3** resulted in more active and low toxic compounds, compared to their precursors **1**–**3** (Table 2).

Among the twelve compounds tested, compound **1a** showed to be the most active one with an IC_{50} = 4.90 μg/mL, but also exhibiting the highest toxicity (CC_{50} = 24.60 μg/mL), with comparable values of potency and cytotoxicity to Amphotericin B (IC_{50} = 4.70 μg/mL; CC_{50} = 25.00 μg/mL), despite the weaker activity when compared to Pentamidine (IC_{50} = 0.44 μg/mL). Besides derivative **1a**, compounds **1c** (IC_{50} = 7.05 μg/mL; CC_{50} = 140.00 μg/mL), **2a** (IC_{50} = 5.05 μg/mL; CC_{50} = 28.00 μg/mL) and **3c** (IC_{50} = 5.94 μg/mL; CC_{50} = 160.00 μg/mL) also showed significant inhibitory properties against promastigote forms of *L. amazonensis*, with a relatively high toxicity observed for **2a**. Considering a security index (SI), expressed by the ratio between cytotoxicity (CC_{50}) and anti-leishmanial potency (IC_{50}), compound **3c** exhibited the best biological profile, with a SI = 26.93, followed by compound **1c** (SI = 19.86) in comparison to all other benzophenone derivatives and to standard drugs Amphotericin B (SI = 5.32) and Pentamidine (SI = 8.68).

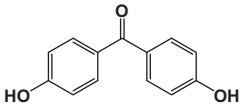
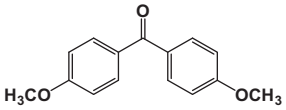
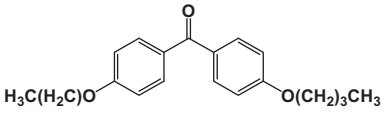
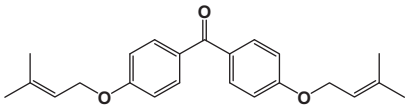
A comparative analysis of biological data set also showed that molecular modifications led to more cytotoxic compounds, as evidenced by almost all monoalkylated derivatives in comparison to the starting hydroxybenzophenones **1** and **2**. As shown in Table 2, except for compound **1c** (CC_{50} = 140.00 μg/mL), all other

Table 2
Chemical structure, biological and Log*P* data for benzophenone derivatives **1**–**3** and **1a**–**3c**, compared to Amphotericin B and Pentamidine

Compound	Chemical structure	IC_{50}[a] (μg/mL)	Cytotoxicity[b]	Log $_P$[c]
1		29.00 ± 6.66	>160.00	3.46
LFQM-115 (**1a**)		4.90 ± 0.72*	24.60 ± 1.94**	3.79
LFQM-116 (**1b**)		9.80 ± 1.83*	40.60 ± 1.85**	5.04
LFQM-117 (**1c**)		7.05 ± 0.44*	140.06 ± 2.80	5.29
2		40.92 ± 1.67	>160.00	3.07
LFQM-118 (**2a**)		5.05 ± 0.21*	28.00 ± 2.08**	3.40
LFQM-119 (**2b**)		8.56 ± 0.18*	28.90 ± 1.50**	4.60
LFQM-120 (**2c**)		7.82 ± 0.50*	87.10 ± 2.52	4.73

(*continued on next page*)

Table 2 (*continued*)

Compound	Chemical structure	IC_{50}[a] (μg/mL)	Cytotoxicity[b]	Log P[c]
3		121.36 ± 6.33	>160.00	2.71
LFQM-121 (**3a**)		21.30 ± 0.70*	116.20 ± 2.18**	3.57
LFQM-122 (**3b**)		23.80 ± 8.90*	>160.00	5.71
LFQM-123 (**3c**)		5.94 ± 1.31*	>160	6.21
Amphotericin B		4.70	25.00	–
Pentamidine		0.44	3.82	–

Values marked with one asterisk means that the derivative differs statistically from their starting compound when p <0.05 by Tukey's test.
Values marked with two asterisk means that the derivative differs statistically from their starting compound when p <0.05 by Tukey's test.
[a] Anti-leishmanicidal activity against promastigote forms of *L. amazonensis*.
[b] Cytotoxicity against murine macrophages.
[c] Calculated lipophilicity expressed by Log$P$ (oct/wat) by using XLOGP3 program version 3.0.1.

monoalkylated derivatives showed a significant increase in cytotoxicity, ranging from 1.8 to 6.5-fold toxic in macrophages when alkyl substituents were introduced in C-4 position of benzophenones **1** and **2**. Probably this observation could be explained by an increase in lipophilicity as a consequence of alkylation of hydroxyl groups, as evidenced by Log$P$ values estimated by XLOGP3 program (Table 2). An increase in lipophilicity could explain the marked increase in leishmanicidal and cytotoxic activity by facilitate the passage of active molecules through the cell membrane barrier. A close relation between lipophilicity and antioxidant activity was also reported by Urzúa and co-workers[16] when they evaluated the antimicrobial activity of a series of spiro-benzofuran derivatives. On the other hand, an increase in molecular volume and lipophilicity seems to have a synergistic important role in cellular selectivity as evidenced by di-alkylated compounds **3a**–**3c** that showed to be active against *Leishmania* with low cytotoxicity. This observation was more evident for compound **3c**, substituted by two prenyl moieties at C-4 and C-4′ position and with a Log$P$ = 6,21, that exhibited the best biological profile, showing the be 20-fold more potent than original benzophenone **3**, against promastigote forms of *L. amazonensis*, along with the lowest cytotoxicity. This compound also showed anti-leishmanial activity comparable to Amphotericin B, despite of the weaker activity related to Pentamidine.

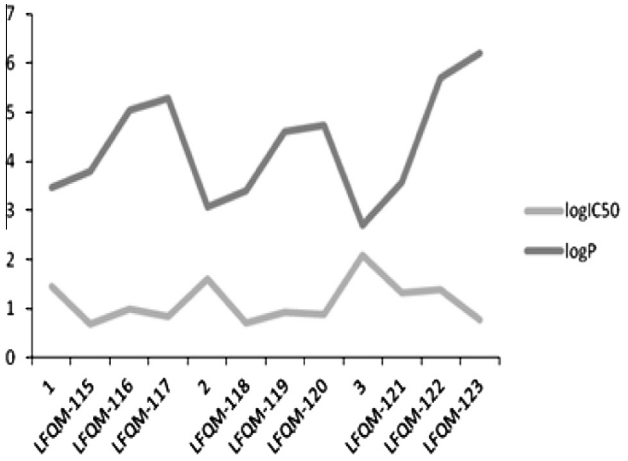


Figure 1. Comparative analysis between variation in Log$P$ and anti-leishmanial activity of compounds **1**–**3** and **1a**–**3c**.

In our study it was evidenced that an increase in the size of side chain substituents led to a proportional increase in lipophilicity and anti-leishmanial potency, as showed in Figure 1.

4. Conclusions

Nine alkylated-benzophenone derivatives (compounds **1a**–**3c**) were prepared in good chemical yields from commercial 2,4-dihydroxybenzophenone (**1**), 4-hydroxybenzophenone (**2**) and 4,4′-dihydroxybenzophenone (**3**). All synthetic derivatives **1a**–**3c** exhibited a remarkable inhibition of the growth of promastigote forms of *L. amazonensis* with the highest anti-leishmanial activity evidenced for compounds **1a**, **1c**, **2a** and **3c**. The most highlighted results seems to be for compound **3c** that showed the best relationship between anti-leishmanial and cytotoxicity against macrophages, with represent a good indicative for therapeutic index for the development of a new drug candidate prototype. In a comparative analysis of all results, it was evidenced that variation in lipophilicity of the target molecules was an important requisite for modulation of antileishmanial activity and toxic effects in macrophages. In early studies, Pereira and co-workers[8] showed that benzophenones with higher lipophilicity present highest activities against *Leishmania* species, which could in fact be related to the ease to come in the macrophage and to reach the parasite. In this context, further studies should be conducted to better understand the mechanism of action of these molecules and their effectiveness against amastigote forms of *Leishmania*. For both, will be used compounds with low cytotoxicity and increased activity in *Leishmania* promastigotes forms. Thus, these series of benzophenone derivatives could be explored as new drug prototype candidates to antileishmanial agents with easy access from commercial starting materials, good lead to new bioactive chemical entities with low toxicity and good selectivity.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.03.045.

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